Correspondence

A colipase fold in the carboxy-terminal domain of the Wnt antagonists – the Dickkopfs

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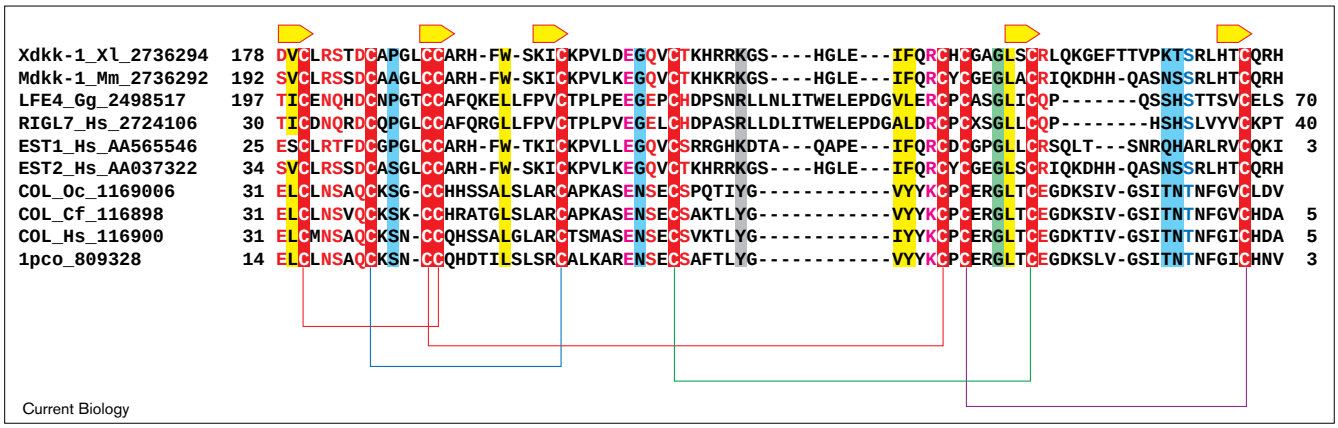
It has recently been shown that a class of secreted proteins — the Dickkopfs — are released by the Spemann organizer of *Xenopus* and are potent antagonists of Wnt signaling [1]. Although it has been reported that the Dickkopf (Dkk) proteins contain two cysteine-rich domains, no direct functional or structural conclusions have been drawn from their sequences. The role of Wnt signaling in animal development has recently become clearer, with the discovery of new components of this pathway [2], and is particularly well illustrated by experiments involving *Xenopus* head formation by the Spemann organizer [2] and wing development in *Drosophila* [3]. These studies led to the characterization of the complete signaling pathway that is initiated extracellularly by the binding of Wnt to the Frizzled-like seven-transmembrane receptors [2], and proceeds intracellularly through a G-protein-coupled cascade [4] and the Armadillo and Lef proteins, which function in the nucleus by regulating transcription [5]. This signaling cascade is necessary in vertebrate development to initiate posterior structures and is antagonized by molecules such as Dkk and Frzb, which are secreted by the Spemann organizer and are required for head induction [6].

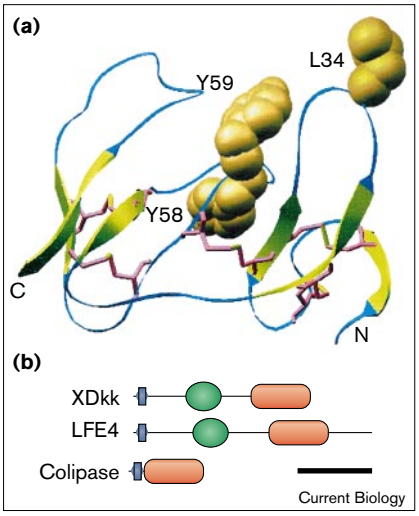
To gain a better understanding of the role of Dkks in Wnt signaling, we carried out a detailed analysis of Dkk sequences and show here that they have a colipase fold. This observation may have direct implications for the function of the Dkks in signaling. It has been noted that the Dkks have two cysteine-rich domains ([6]; see also below), and we found additionally that the carboxy-terminal domain has the pattern of cysteines typical of the colipase domain (Figure 1). An iterative database search of the non-redundant protein database at the National Center for Biotechnology Information (NCBI) using the PSI-BLAST program [7], with the Dkk sequences as queries, recovered the colipases from different mammals at a statistically significant expectation (*e*) value of 10^{-4} in the second iteration. This was confirmed by reverse searches using different colipases, which retrieved the Dkks at similar *e* values within three iterations. Multiple alignment based secondary structure prediction using the PHD program [8] and subsequent threading [9] through the

Figure 1



Alignment of the Dkks with colipases and other related molecules. The alignment was constructed using the CLUSTALW program [14] and modified on the basis of the results of the PSI-BLAST searches. Xdkk-1 and Mdkk-1 are the Dkks from *Xenopus laevis* (Xl) and *Mus musculus* (Mm) respectively; COL stands for the colipases. LFE4 and RIGL7 are secreted molecules of unknown function; 1pco indicates the porcine colipase for which the crystal structure has been determined. The species abbreviations are: Cf, *Canis familiaris*; Hs, *Homo sapiens*; Oc, *Oryctolagus cuniculus*; Gg, *Gallus gallus*. The Genbank gene identification numbers, or accession numbers in the case of the two human expressed sequence tags (ESTs), are shown after each sequence. The numbers flanking the alignment are the numbers of the residues on either side of the aligned domain in the protein. The conserved residues were colored according to the 85% consensus rule: polar residues, red; acidic and basic residues, pink; hydroxylic residues, blue; hydrophobic residues, yellow background; small residues, blue background; tiny residues, green background; large residues, gray background. The conserved cysteines, which form the disulfide-bonding pattern typical of this family, are shown in inverse red shading. The disulfide-bonding network connecting the cysteines is shown in a separate color for each pair. The predicted structural elements based on the porcine colipase crystal structure are shown above the alignment, with the arrows representing β elements.

Figure 2



(a) A three-dimensional model of the colipase fold on the basis of the porcine colipase crystal structure (protein database PDB code 1pco). The β strands are shown in yellow, the loops in blue and the disulfide bonds in pink. The hydrophobic residues (in the single-letter amino-acid code), which are referred to in the text and are possibly involved in lipid interaction, are shown as space-filling spheres in gold. N, amino terminus; C, carboxyl terminus. **(b)** The domain organization of the colipase-domain-containing proteins. The proteins are drawn to scale and the domain lengths are defined on the basis of sequence similarity. Blue, signal peptide; green, amino-terminal cysteine-rich domain; red, colipase domain; thick bar, 100 amino acids.

protein structural database (PDB) suggested the existence of a similar fold in the carboxy-terminal domain of the Dkks and the colipases. On the basis of these observations, we predict that the disulfide-bonding pattern in the Dkks is the same as in the colipases (Figure 1).

The colipase fold (Figure 2a) is typical of a wide range of small proteins, which are involved in a number of specific protein–protein interactions and include, in addition to the colipases, scorpion and snake toxins and protease inhibitors [10]. These proteins have a series of short β strands with large connecting loops, which are held together by disulfide bonds. This results in a flat fold with finger-like protuberances, which act as interactive surfaces [11]. The colipases bind to the pancreatic lipases and act as cofactors for them in lipid hydrolysis [11]. It has been shown that the colipases are necessary for the interactions of the lipases with hydrophobic lipid micelles during the process of lipid digestion, and the exposed hydrophobic residues in the finger-like elements are involved in this lipophilic interaction [12,13]. Several of these hydrophobic positions are conserved between the carboxy-terminal domain of Dkk and the colipases (Figures 1, 2b), suggesting that Dkk — like the colipases — interacts with lipids. One direct functional implication of this observation is that the colipase-like domain of Dkk may be necessary for the membrane association of this protein, which in turn may be required for the inhibition of Wnt secretion or Wnt–receptor interaction. We thus propose a testable hypothesis that the inhibition of the Wnt function by Dkk is closely associated with the cell membrane and that the carboxy-terminal, colipase-like domain of Dkk mediates this association. The amino-terminal domain of Dkks may directly interact with Wnt or its receptor, Frizzled. The Wnt inhibition by Dkk thus appears to be distinct from the inhibition mediated by Frzb, which is a dominant-negative form of Frizzled ([2] and references therein).

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Protein folding: Think globally, (inter)act locally

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Groß [1], in his account of the paper by Plaxco *et al.* [2], endorses the claim that the protein folding field is split into two camps over the issue of the importance of local versus long-range interactions in folding kinetics,

with the opposition to local interactions being led by us. This is incorrect, resulting from a confusion by Groß and Plaxco *et al.* of two different topics. Plaxco *et al.* [2] have made the interesting proposal that the rate of folding of two-state proteins roughly correlates with contact order; the greater the proportion of local contacts (the lower the contact order), the faster the folding. We have discussed the parallel, but different, issue of the implications of the stabilization by local versus non-local interactions on thermodynamic stability *and* folding kinetics of proteins [3,4].

Our argument, in a nutshell, is that if a local element of native structure is hardly populated in the denatured state but is present in the transition state, then stabilizing that element will speed up folding and stabilize the protein. But if that element is stabilized to such an extent that it becomes highly populated in the denatured state of the protein, then the stabilization energy of that element of structure does not contribute to the overall free energy of folding, and further stabilization of that structure does not increase the rate or stability of folding. In addition, excessive stabilization of the denatured state leads to the accumulation of intermediates and the kinetics of folding deviating from the two-state systems that were analysed by Plaxco *et al.* [2]. There has to be a mix of short-range and long-range interactions for a protein to form a stable, kinetically accessible fold. Our predictions have been verified experimentally by Serrano and colleagues, who have engineered the stability of α-helices in three proteins and measured the effects on folding stability and kinetics [5,6].

As noted by Plaxco *et al.* [2], the correlation between contact order in the native structure and the folding rate is consistent with the nucleation-condensation mechanism, which we had earlier proposed [4,7,8]. One of the postulates of the nucleation-condensation mechanism is that "any potential nucleation site that is composed mainly of adjacent residues should be just weakly populated in the denatured state and become structured only in a high energy intermediate or transition state when it is stabilized by interactions elsewhere in the protein" [4]. This clearly does not conflict with any proposed importance of contact order, but does imply that the contact order becomes important in the transition state, rather than the denatured state.

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Introns

The agony column

If you have a problem, no matter how large or small, Current Biology*'s 'agony aunt' Amber can help.*

Dear Amber,
My lab head doesn't appreciate me and thinks it's my fault when the experiments don't work. How can I make him realize his error?

Wounded, Uppsala, Sweden

Dear Wounded
Long experience has taught your lab head that it probably is your fault when the experiments don't work. (Of course, this is no excuse if your lab head doesn't have long experience. But it's probably true nonetheless.)

Amber

Dear Amber,
Genetic knockouts in mice are essential to my research on nerve growth factors and I'm seeing some very interesting brain defects and behavioural abnormalities in my early experiments. But mouse costs at my institution are exorbitant and, as I don't have Howard Hughes funding, my ability to do experiments is severely restricted. Do I have any option other than to move?

Despairing, Cambridge, USA

Dear Despairing
Here's a money-making idea for you. Cat owners are often pilloried for the damage their cats inflict on wildlife and might be willing to pay well for alternative ways to exhaust the natural hunting instincts of their pets. Brainless mice sound like an ideal solution, and their sale could defray a portion of your costs.

Amber

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